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Cutaneous delayed-type hypersensitivity reactions in smokers with chronic bronchitis and recurrent exacerbations: comparison with asymptomatic smokers and never-smokers

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The aim of the present study was to investigate whether smoking patients with chronic bronchitis (CB) and recurrent exacerbations show signs of depressed cell-mediated immunity (CMI), as reflected in the cutaneous delayed-type hypersensitivity (DTH) reaction, in comparison with asymptomatic smokers and healthy never-smokers.

The study was a comparative clinical study performed at a university hospital center of respiratory medicine. Sixteen smokers with stable CB and recurrent exacerbations, five of whom had mild airflow obstruction, 18 asymptomatic smokers and 18 healthy never-smokers, all aged between 35 and 64 years, participated. No subjects treated with corticosteroids or *N*-acetylcysteine were included. Cutaneous DTH-reactions to seven recall antigens were assessed with Multitest®, a standardized *in vivo* test of clinical CMI. Reactions were assessed 48 h after application by measurement of skin induration. A score (sum in mm of positive reactions) was created to assess overall reactivity.

Neither the score nor the number of positive reactions differed significantly between the three study groups. Men had a significantly higher reactivity than women ($P<0{\cdot}05$) irrespective of group affiliation. No influence of smoking status on DTH reactivity could be seen. In the CB group no correlation was found between DTH reactivity and number of exacerbations the past 2 years. Patients with chronic bronchitis and recurrent exacerbations did not differ from asymptomatic smokers or healthy never-smokers with respect to cutaneous DTH reactions. Depression of CMI, as measured in this study, does not seem to be a primary factor behind recurrent exacerbations in smokers with CB.

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Introduction

Tobacco smoking induces profound immunological and inflammatory changes, both locally in the airways and systemically (1). Smoking impairs host defences and increases susceptibility to infection (2,3), but the reason why only some smokers develop problems with repeated respiratory tract infections is largely unknown. The disposition for airway infections seen in some smokers seems to be related to the presence of chronic bronchitis (CB) (4). The predominant manifestation of infection in patients with CB is the exacerbation where a bacterial or viral cause can be demonstrated in the majority of cases (5–7). These exacerbations occur recurrently and often result in repeated courses of antibiotic treatment and hospital admissions (8,9).

Impairment of cell-mediated immunity (CMI), induced by smoking or by other mechanisms, is one of several possible explanations for the increased susceptibility to bronchial infections in this patient group. Different aspects of CMI have been investigated previously in patients with CB or chronic obstructive pulmonary disease (COPD) and recurrent exacerbations. Reports of decreased blood monocyte chemotaxis (10) as well as defective monocyte candidacidal ability (11) have been presented. In addition, decreased phagocytosis (12) and candidacidal ability (13) of neutrophil granulocytes have been reported. Depressed mitogen- and antigen-stimulated proliferation of blood lymphocytes has also been described (14).

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As an *in vivo* measure of clinical CMI, the delayed-type hypersensitivity (DTH) reaction to different recall antigens has been used. The role of smoking for DTH reactivity is not known, but several researchers have described decreased DTH reactions indicative of defect CMI in patients with CB (12,13,15). In these studies, most patients were in an advanced stage of disease with significant chronic airflow obstruction. This could be of importance since malnutrition with low body weight, a condition strongly associated with depressed DTH reactivity (16,17), is common in this patient group (18–20). The question of whether the depressed DTH reactivity was a primary phenomenon connected with recurrent infections and the development of chronic airflow obstruction, or only secondarily associated with an advanced disease stage, was not addressed in these studies.

The aims of the present study were to investigate whether patients with CB and recurrent infectious exacerbations without advanced disease show a lower reactivity to a standardized DTH test compared to smoking and non-smoking controls, and to analyse the possible influence of smoking *per se* on systemic recall immunity as reflected in DTH-reactivity. To control for the possible confounding influence of malnutrition on DTH reactivity, calculation of body mass index (BMI) was done in all participating subjects.

Materials and Methods

DESIGN

Delayed cutaneous hypersensitivity to seven recall antigens was studied in three well-defined study groups: patients with CB and recurrent exacerbations, asymptomatic smokers and healthy never-smokers. The study forms part of a comprehensive investigation, exploring different aspects of airway and systemic immunity in patients with CB and recurrent exacerbations and asymptomatic smokers, parts of which have been presented previously (21). The third study group of healthy never-smokers was added after the completion of the study of the two smoking groups. Identical methods and criteria for inclusion and exclusion of the subjects were used.

The subjects were recruited from patient files at the department of respiratory medicine and by advertising in a daily newspaper. A medical examination and a lung function test took place at visit one. Height and weight for calculation of BMI ($BMI = weight \times height^{-2}$) were registered. At a second visit within 2 weeks thereafter, a test for cutaneous DTH reactions was performed and was evaluated 48 h later.

The study was approved by the ethics committee at the University of Göteborg. The subjects gave their written consent after both written and oral information about the study.

SUBJECTS

Two control groups, each with 18 subjects, were studied. The first was composed of healthy never-smokers (NS) with normal lung function defined as forced expiratory volum in the first sec (FEV_1) >80% of predicted (pred.) norma value based on gender, age and height. Asymptomati smokers (AS) with normal lung function as defined abov made up the second control group. They were all curren smokers, having smoked for more than 10 years anc consuming at least 10 cigarettes per day without fulfilling the American Thoracic Society (ATS) criteria of chronic bronchitis (22).

The patient group was composed of 16 subjects with symptoms of CB as defined by ATS (22), i.e. chronic or recurrent productive cough on most days for a minimum of 3 months per year during the past 2 years. Co-existing chronic airway obstruction defined as FEV_1 <80% pred. was allowed. All were current smokers, having smoked for more than 10 years and consuming at least 10 cigarettes per day. All had a history of two or more acute exacerbations during the past 12 months as defined by Boman *et al.* (23), i.e. mucopurulent or purulent sputum and an increase (compared to usual) in one of the following symptoms: cough, sputum volume, breathlessness, difficulty of expectoration. The total number of exacerbations during the past 2 years was recorded.

Subjects between 35 and 65 years of age were chosen for the study.

Criteria for exclusion were: treatment with *N*-acetylcysteine (NAC), antihistamines or antibiotics, vaccination or other immunomodulating treatment within 4 weeks prior to the first investigation, glucocorticosteroid treatment (oestrogen included), whether local or systemic, or other immunosuppressant treatment within 3 months prior to the first investigation, and symptoms of infectious respiratory disease within 4 weeks prior to the investigation.

Subjects were also excluded if they had: a baseline FEV_1 <45% pred., a post-bronchodilator increase in FEV_1 >15% pred., abnormal chest radiograph, other active pulmonary diseases such as sarcoidosis, cystic fibrosis, α_1-antitrypsin deficiency or a history of asthma, known immunodeficiency, significant skin diseases, e.g. contact allergy, eczema or atopy and other concurrent severe diseases.

LUNG FUNCTION TEST

Ventilatory lung function (FEV_1% pred.) was measured with a Vitalograph Alpha (Vitalograph Ltd, Buckingham, U.K.) in a standardized manner according to the directions of the European Coal and Steel Community (24).

DELAYED-TYPE HYPERSENSITIVITY TEST

A cutaneous DTH test (Multitest®CMI, Pasteur Mérieux, Lyon, France) was applied at visit two. The Multitest system consists of a plastic disposable multipuncture device, by which low concentrations of seven glycerinated antigens (tetanus, diphtheria, *Streptococcus*, tuberculin, *Candida, Trichophyton* and *Proteus*) and a glycerinated negative control are administered simultaneously by prick-puncture. The inner surface of one of the forearms was used.

TABLE 1. Demographic and clinical data

	Never-smokers	Asymptomatic smokers	CB with exacerbations
n	18	18	16
Age (years)	46 (36–64)	45* (35–58)	53 (38-63)
BMI (kg m^{-2})	23 (20–38)	24 (20–32)	25 (23–35)
FEV_1% pred.	105†,‡ (93–136)	93 (81–114)	88 (62–124)
Pack years	0	28 (15–57)	29 (16–44)
Current smoking, cigarettes day^{-1}	0	20 (10–30)	20 (10–30)
Male/female	8/10	7/11	4/12
Duration of CB (years)	0	0	8 (4–26)
Exacerbations in 2 years (*n*)	0	0	7 (4–12)

Data are presented as median values with range in parenthesis. CB: chronic bronchitis; BMI: body mass index.
*asymptomatic smokers *vs.* CB group, $P<0.05$; †never-smokers *vs.* asymptomatic smokers, $P<0.05$; ‡never-smokers *vs.* CB group, $P<0.001$.

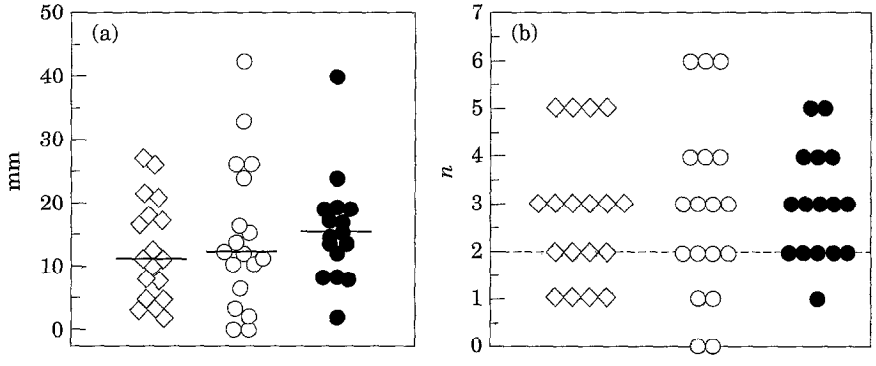

FIG. 1. (a) Median and individual scores (sum in mm of positive cutaneous reactions) in DTH test. (b) Individual number of positive DTH reactions. The dashed line marks the upper limit of hypoergic responses. ◇, Never-smokers; ○, asymptomatic smokers; ●, chronic bronchitis patients.

One investigator performed all applications according to the instructions of the manufacturer, and the reactions were assessed 48 ± 4 h later by the same investigator. Reactions were assessed by measuring the skin induration in two perpendicular diameters. A skin induration with a mean of the two diameters of ≥2 mm was considered to be a positive reaction.

The definitions of hypoergy/anergy and the scoring system for evaluation of overall reactivity proposed by Kniker *et al.* (25) were used. Accordingly, the total score for each individual was defined as the sum in millimeters of all positive reactions, and hypoergy as fewer than three positive reactions. Anergy was defined as no positive reaction.

STATISTICAL CONSIDERATIONS

A StatView® 4.5 (Abacus Concepts, Berkeley, CA, U.S.A.) software package was used for the statistical analysis. As most of the data did not show a normal distribution, data are presented as median and range unless otherwise stated. For comparisons between groups, a Kruskal–Wallis test was performed, followed by Wilcoxon rank-sum tests with Bonferroni corrections for multiple comparisons when appropriate. *P*-values <0·05 were accepted as significant. A Spearman rank correlation coefficient was calculated to investigate correlations between clinical variables.

Results

A total of 52 subjects (18 NS, 18 AS and 16 CB patients) were enrolled and completed the study. All subjects were Caucasian and from the west coast of Sweden. Subject demographic and clinical data are presented in Table 1. BMI did not differ significanly between the three study groups and there were no individuals with a low BMI (normal range 19–25) (26).

The number and size of positive reactions to the seven different antigens of Multitest® CMI are presented in Table 2. The three antigens with the largest number of positive reactions were tuberculin, tetanus and diphtheria in all three groups. In order to analyse whether reactivity to the different individual antigens differed between the groups, the number and size of reactions to antigens with a

TABLE 2. Number and size of positive reactions to seven antigens in Multitest®

Antigen	Never-smokers (n=18)			Asymptomatic smokers (n=18)			CB with exacerbations (n=16)		
Tetanus	14	6·25	(2–12·5)	14	7	(3·5–10·5)	11	7·25	(3·5–13)
Diphtheria	7	4·5	(2–9·5)	8	4	(2–11)	8	5	(2·5–12·5)
Streptococcus	1	2·5	—	4	2·5	(2–4)	2	2·5	(2–3·25)
Tuberculin	9	4·5	(2–8·5)	15	4	(2–14·5)	13	4·5	(4–9·5)
Candida	2	3·0	(3)	3	4·5	(2·5–9)	6	3	(2–3·75)
Trichophyton	3	3·0	(2–3)	3	4	(2·5–4·5)	0	—	—
Proteus	5	3·5	(2–5)	5	3	(2–3·5)	8	3	(2–4·5)

Sizes in mm; median values are shown with ranges in parenthesis. CB: chronic bronchitis.

positive reaction in five or more subjects in each group (tetanus, diphtheria, tuberculin and *Proteus*) were compared between the groups. No significant differences were found.

The individual scores and number of positive reactions in each individual are presented in Fig. 1(a) and (b). There were no significant differences between the groups in DTH score or in number of positive reactions. The median scores were 11, 12·25 and 15·25 mm, in the NS, AS and CB groups, respectively. The median number of positive reactions was 3 in all groups. Hypoergy was found in 8/18 (44%), 8/18 (44%) and 6/16 (38%) patients in the NS, AS and CB groups, respectively. Two subjects in the AS group were anergic.

Five subjects in the CB group had mild airflow obstruction (FEV_1% pred. median 71, range 62–79). They did not differ significantly from the non-obstructive subjects with respect to age, sex, smoking history, duration of CB or number of exacerbations. Their median DTH score was lower than that of the other 11 CB patients (15 *vs.* 17·5 mm), but the difference was not significant. They also had a slightly lower median BMI than that of the non-obstructive patients (24 *vs.* 27) but this difference was not statistically significant.

A significant difference ($P<0·05$) in DTH score was noted between the male and female subjects in the total population with a median score of 17·5 mm in men and 11·5 mm in women. As men and women were unevenly distributed between the study groups, a comparison of each sex separately was performed. No significant difference between the groups was found for either sex.

In order to investigate the relationship between DTH reactivity and clinical variables in the CB group, a Spearman rank correlation coefficient was calculated between the results of the skin test, using both score and number of positive antigens and 1. the number of exacerbations during the past 2 years and 2. FEV_1% pred. No significant correlations were found.

MULTIPLE LINEAR REGRESSION ANALYSIS

To analyse further the relative influences on the DTH score of gender, smoking status, BMI and age, these variables were entered into a multiple regression model as independent variables with DTH score as the dependent variable. The results are shown in Table 3. The R^2-statistic for the model was 0·201, i.e. 20% of the total variation in DTH score was explained by the model. Gender had a statistically significant influence on DTH score whereas no significant influence could be demonstrated for either smoking, BMI or age.

Discussion

In the present study, we investigated one aspect of cell-mediated immunity, the cutaneous DTH reaction to recall antigens, in a well-defined group of patients with CB and recurrent exacerbations compared with asymptomatic smokers and healthy never-smokers. We found no sign of depressed DTH reactivity in our patient group when compared with either of the control groups and we could not demonstrate any significant influence of smoking, BMI or age on DTH reactivity. We found a significantly lower reactivity in women than in men.

The rationale to study DTH responses in CB patients with recurrent exacerbations is based on results from previous studies (from the fields of oncology and surgery) where a reduced DTH response was found to be indicative of a clinically significant impairment of CMI (27,28). The DTH reaction represents a combination of immunological

TABLE 3. Multiple linear regression of DTH score on gender, smoking status, BMI and age

Variable	Coefficient	SEM	CI	P-value
Gender	7·25	2·59	(2·05; 12·45)	0·007
Smoking status	3·73	2·60	(−1·51; 8·97)	0·16
BMI	0·41	0·31	(−0·21; 1·04)	0·19
Age	−0·09	0·17	(−0·42; 0·25)	0·59

DTH: delayed-type hypersensitivity; BMI: body mass index; SEM: standard error of the mean; CI: 95% confidence interval.

reactions that can be studied *in vitro* including lymphocyte transformation and proliferation in response to specific antigen stimulation, lymphokine production and monocyte migration. Although it must be emphasized that the DTH reaction does not give complete information on the immunological mechanisms involved in cell-mediated immunity, a correlation between low DTH reactivity and reduced lymphocyte reactivity *in vitro* upon stimulation with phytohaemagglutinin (PHA) or tetanus has been found in several studies (13,29,30). In addition, an association between low DTH reactivity and reduced blood neutrophil and alveolar macrophage candidacidal ability has been described (15).

The Multitest® CMI system used in the present study of DTH reactivity tests the capacity of the individual to mount a cell-mediated immune response to seven recall antigens. It is assumed that the antigens are common in the environment and that most adults have already been exposed to them, either via childhood immunization or natural exposure. The reliability and reproducibility of the test has been demonstrated previously (25,31,32). Several studies have indicated that gender is important in determining responsiveness (with smaller responses in women) (33,34), an observation which is confirmed by our results. Data on the effect of increasing age are somewhat conflicting but most studies indicate reduced responses in ages over 70 years (29,30,33,35). Apart from age and gender, a number of other factors may influence DTH reactions, such as immunosuppressive therapy, malnutrition (16,17), recent infection (31), surgical trauma (36) and malignancies (37). As to the possible effect of smoking, the response to tuberculin was investigated in smokers and non-smokers by Onari *et al.* (38) with no differences found between the groups. In the present study, the inclusion and exclusion criteria were chosen to minimize undesired influences on the test result. In spite of this, multiple regression analysis of DTH score showed that only a relatively small fraction of the variation could be explained by registered factors.

Based on previous studies, we hypothesized that DTH reactions would be lower in the group with CB and recurrent exacerbations but found no support for this hypothesis. Instead, the mean size of positive reactions was largest in the CB group although this difference was small. Our CB group was dominated by women, known to have a lower reactivity than men. Hypothetically, an equal distribution of men and women in all three study groups might have resulted in a higher DTH reactivity in the CB group.

Our results differ from those of several previous studies where depressed DTH reactions have been reported in CB and COPD patients with a history of recurrent exacerbations (12,13,15). Hypothetically, smoking status could be of importance for DTH reactivity. In the referred studies smoking history is either not reported or not comparable between patient and control groups (12,13,15). The design of the present study with two control groups, one with similar smoking history and present smoking habits to those of the CB group and one with never-smokers, enabled us to analyse the influence of smoking *per se* on DTH reactivity. Our results, however, indicate that smoking has no depressive effect on DTH reactivity, and consequently that this cannot explain the differencies between the present and the previous studies.

There are other discrepancies in patient selection which might explain the differing results. Firstly, there are differences in disease stage. Thirty-one per cent (5/16) of our CB patients had airflow obstruction. In one of the earlier reports (12), the fraction of patients with airflow obstruction was similar (31%), whereas in the other two reports (13,15), all (100%) patients had airflow obstruction. Furthermore, where reported (13), the degree of airflow obstruction was higher than in our CB patients. This would seem to indicate an association between impaired DTH reactivity and a more advanced state of inflammatory airways disease with airflow obstruction, an association not discernible in our patients with no or mild airflow obstruction. There is, however, another factor associated with severe airflow obstruction that might influence DTH reactivity. Body weight was strongly related to FEV_1 in a large study of COPD patients by Wilson *et al.* (19) and malnutrition is a common condition in these patients (16,20). Furthermore, depressed DTH reactivity is a common feature of malnutrition (16,17). Although we have no knowledge of the nutritional status of the subjects in the referred studies, it is reasonable to speculate that the depressed DTH reactivity found in these patients with a more advanced disease stage could coincide with a state of malnutrition. In our patients with no or mild airflow obstruction and with normal or high BMI in all cases, there is no such connection.

Secondly, age might be of importance. All participants in our study were between 35 and 65 years, a range where DTH reactivity can be expected to be relatively constant. The minimal influence of age on DTH score in our subjects would seem to support this supposition. In the referred studies however, mean ages were close to or above the upper limit of this interval, where relatively small differences in mean age between patient and controls groups might be of importance. Whatever the cause might be for the previous findings of depressed DTH reactivity in CB and COPD patients, it can be of clinical relevance. However, it seems to be related to advanced disease stages, and not primarily to smoking.

To conclude, we found no sign of impaired cell-mediated immunity by one measurement in smoking patients with chronic bronchitis and recurrent exacerbations. This result gives no support to the hypothesis that depressed cell-mediated immunity is a primary factor behind recurrent exacerbations or the progression to chronic airflow obstruction in this patient group.

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